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BIOLOGICAL ACTIVITY OF THE THERAPEUTIC HERB GERANIUM WALLICHIANUM FROM KASHMIR'S HIMALAYAN REGION

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ABSTRACT

The biological activity analyses of the crude extracts of Geranium wallichianum roots and leaves are presented in this work. To investigate the therapeutic potential of this significant plant, the crude extracts were separated into n-hexane, the ethyl acetate, and methanol fractions. These fractions were then exposed to various biological activities. The findings showed that in various tests, the natural extracts and various root and leaf fractions exhibited varying degrees of antibacterial and antifungal activity. In a variety of tests, the root extract and its various components demonstrated generally higher activity. Additionally, GC-MS was used to assess the bioactive ingredient.

KEYWORDS: GC-MS, antifungal, antibacterial, and Geranium wallichianum.

INTRODUCTION

Among the most important sources of medications are plants. Most of the pharmaceuticals employed nowadays originate from plants. Plants with medicinal properties are the primary source of metabolites that are used as substances and aromatic compounds of beneficial

importance. The safety, affordability, effectiveness, and ease of accessibility of medicinal plants are significant benefits for their therapeutic applications in a variety of illnesses. Traditional medical professionals were compelled to use medicinal plants extensively in their daily practices due to their many benefits (Nawed Anjum et al., 2015).

For thousands of years, plants for medicinal purposes have been an essential component of human healthcare, and they are the basis of traditional medicine in many different cultures. These medicinal plants are prized for their ability to avoid treat, or lessen the symptoms of a wide range of illnesses. The treatment of herbs has been used for centuries; it is mentioned in ancient manuscripts such as the Egyptian Ebers Papyrus, the Indian Rig-Veda, and the Greek works of Hippocrates. This makes it one of the earliest types of healing. Due to their medicinal qualities and antimicrobial activity, plants the oldest human companions have long been the focus of academic studies (Ghorbanzadeh HR et al., 2019).

Humankind has been employing remedies to enhance their health and treat ailments for as long as they have existed on Earth. Plants were the primary and significant source of prevention and therapy in the sixteenth century until the development of iatrochemistry (Kelly K et al., 2009). There hasn't been a review of Kashmir's traditional medicinal plant applications, and when it has, it has either concentrated on certain populations or geographical areas. Many nations employ plants as medicine since they are a source of strong and effective medications (Srivastava, J. et al. 1996).

Graw medications use a variety of medicinal plant parts, including roots, stems, fruit, flowers, twigs, exudates, and modified plant organs, which have a range of therapeutic qualities. Furthermore, traditional medicines have proliferated globally, and many pharmaceutical and conventional medications are directly drawn from nature. Nearly 80% of people on the planet today, who reside in underdeveloped nations, rely on conventional herbal remedies as their main source of healthcare. Many of the herbal medications used in traditional medicine are not well understood and have not been put to the test using scientific methods. (Qazi, M. et al., 2016 and Ekor, M. et al., 2013).

The need for well-liked herbal goods is frequently not met by traditional sources of medicinal plants. Many species' populations are restricted in their natural environments, necessitating conservation measures to preserve them. (DK Ved and others, 2008).

More than 90% of these medicinal plants in India are gathered from wild sources in an unplanned, unscientific, and unregulated manner, resulting in a supply that is unstable, exploitative, and unsustainable. Additionally, locals are ignorant of morphological characteristics, which frequently results in the purposeful adulteration of various products (Schippmann et al., 2006).

In accordance to a current World Health Organisation definition, conventional healthcare (including herbal medications) includes methods of treatment that have been used for a hundred years or more before modern medicine developed and expanded, and are still used today (WHO 1991). Only traditional medicines that primarily employ the processing of medicinal plants for therapeutic purposes are considered herbal drugs. Their usage in Indian, Chinese, Egyptian, Greek, Roman, and Syrian texts is documented as early as 5000 years ago. The Rig-Veda, the Atherveda, Charak Samhita, and Sushruta Samhita are examples of classical Indian texts. Therefore, the traditional medicines and herbal remedies have been derived from scientific heritage and the rich traditions of old civilisation. In (Kamboj VP et al., 2000).

Recognised locally as "Ratanjot" in Kashmari and frequently referred to as "Shephred's needles," Geranium wallichianum D. Don is a member of the Geraniaceae family (Kamboj VP et al., 1976). This perennial herb has a robust, hairy stem that is approximately 1-4 feet long, thick rootstock, and blue blooms that range in diameter from 3 to 8.5 cm. It is clear that the herb has a significant amount of the genus' astringent qualities. The root supply is used to cure toothaches and eye problems (G. Watt et al., 1972). The plant's roots are used to treat leucorrhea, diarrhoea, dysentery, mouth ulcers, and passive haemorrhage (Z. K. Shinwari et al., 2003).

MATERIAL AND METHODS

Plant material: The plant Geranium wallichianum was collected from budhnambal area of Kupwara district, in 15 September 2023 (Timing 12.04pm) and was identified, Department of Botany, centre of biodiversity (CBT) University of Kashmir. A voucher specimen number 9043-KASH specimen has been deposited at the herbarium of the Botany Department, University of Kashmir.

Extract preparation: The powdered rhizomes of the plant (2.72g) were macerated in methanol, ethyl acetate and hexane (12 L), for 24 hours at room temperature and filtered. The procedure was repeated 3 times. All the three filtrates were combined and concentrated under vacuum at 40◦C using rotary evaporator.

Antibacterial activity: In this assay, strains of Salmonella typhi, Escherichia coli, Staphylococcus aureus, Proteus, and Cons were employed. After cooling to 45°C, 60 mL of molten agar was combined with 0.6 mL of the test organism's broth culture employing a single sterile pipette. The mixture was thoroughly mixed and then transferred into a sterilised petri dish (0.2 mL of the culture itself was added to 20 mL of agar for the 9 cm petri dish). For every organism, duplicate plates were made. In order to ensure correct distribution of the wells in the centre and the perimeter, a sterilised metallic cork borer was used to dig the necessary number of wells in the medium while the agar mixture was given time to set and harden. Agar plugs were then removed. 50 µL of each dispersion was added to the corresponding well after the standard solutions of the test substances at an amount of 10 mg/mL were made in sterile DMSO. After allowing diffusion at room temperature, the plates were incubated for 24 hours at 37°C. The well size was also recorded, and the circumference of the regions of inhibition was determined to the closest millimetre.

Antifungal activity: In this investigation, trains of Alternaria solani, Fusarium oxysporum, and collectotrichum were employed. The food poisoning approach was used to assess the extracts' antifungal activity. To cultivate fungus in the lab, potato dextrose agar medium was created. Petri dishes of the conventional size (100 x 15 mm) are needed for the entire experiment. PDA was prepared by mixing 39 grammes of PDA powder with 1000 millilitres of water that was distilled and stirring the mixture until it was homogenised. The PDA combination was then autoclaved for 15 minutes at 121°C and 15 psi of pressure to sterilise the media. The culture mixture was then divided into 20 ml portions per petri dish and placed partially covered on a laminar circulation of air to allow the agar to cool and harden at room

temperature. According to Shrestha and Tiwari, Zaker et al., and Mohammad et al., the poisoned food technique was used for the antifungal assay. With minor adjustments. Sterilised petri plates were filled with 2 mL of every plant concentration, followed by 20 mL of sterile melted PDA and gentle circular stirring to ensure homogenisation. With control, the identical process was carried out. Every petri dish was given time to solidify. A sterile 5 mm dimension cork borer was then used to create a 5 mm diameter disc from the progress of the 7-day-old culture. Every disc was aseptically positioned in the middle of each treatment-containing petri plate. After being taped shut, the inoculated plates were left to incubate for seven days at room temperature. Three duplicates of each treatment were made. The Vernier calliper was used to measure the pathogen's growth.

Gas chromatography – mass spectrometry analysis (GC-MS): A GC-MS was used for identification and separation. Thermo Scientific's Trace GC Ultra/ISQ Single Quadruple MS and TG-5MS fused silicon capillaries column (30 m, 0.251 mm, 0.1 mm film width) were utilised for the GC-MS study. Helium gas was utilised as a carrying gas at a steady flow rate of 1 mL/min for GC-MS detection, and an electron ionisation apparatus with ionisation energy of 70 eV was employed. The analysis takes into account a sample injecting volume of 1 µL. The temperature of the MS transfers line and injector was fixed at 280 °C. The temperature of the oven was set to start at 40 °C (hold for 3 minutes) and increase by 5 °C each minute (hold for 5 minutes) to 280 °C as the ultimate temperature. Using a percentage of the relative peak area, the quantification of each detected component was examined. Comparing the compounds' relative retention times and mass spectra with the GC-MS system's NIST and Wiley library data allowed for a provisional identification of the substances.

RESULT

Preparation of Plant Extracts: Different plant extracts were prepared from collected plant parts using solvents (methanol, ethyl acetate, and hexane) of different polarities. The dried plant extracts were weighed and summary of extraction yield is shown in table 1. The extractive yield in ethyl acetate extracts varied from 10.93% to 14.4% and 12.41% to 15.80% in methanol extract, while hexane extracts, the range was from 10.85% to 11.51%.

Table 1: Summary of the Extraction Yield (%) of Collected Used Parts.

Extractive Yield (% w/w) Type of Extract				
Sr. No	**Botanical name/part used**	**Methanol**	**Ethyl acetate**	**Hexane**
1	**Geranium wallichianum Roots**	12.41	10.93	11.51
2	**Geranium wallichianum leaves**	15.80	14.4	10.85

Antibacterial Effect with Concentration of Plant Extracts of different Test Pathogens: In vitro antibacterial activity of aqueous, ethanol and acetone extract of Geranium wallichianum rhizome and Geranium wallichianum were recorded against S. aureus, salmonella, E. coli, proteus, cons spp. as shown in table 2. Bacterial activity of G. wallichianum Roots, hexane extract shows best antibacterial activity against bacteria such as the bacteria are S. aureus, and E. coli, spp. G. wallichianum leaf extract exhibits stronger antibacterial action against E. coli and salmonella G. wallichianum Roots ethyl acetate exhibits strong antibacterial actions against cons. Gram-negative bacteria was shown to be more vulnerable to the antibacterial activity of hexane and methanol extracts of G. wallichianum Roots and leaves. Plant extracts was further selected to test their antifungal activity figure 1.

Table 2: Effect with Concentration of Plant Extracts of different Test Pathogens.

Plant		**Geranium Wallichianum Roots**			**Geranium Wallichianum leaves**		
Zone of inhibition							
Test pathogen	**Conc. Mg/ml**	**MeOH**	**EtOAc**	**hex**	**MeOH**	**EtoAc**	**Hex**
S. aureus	10	-	10	18	15	-	14
Salmonella	10	-	12	14	18	20	12
E. coli	10	-	12	18	20	21	13
Proteus	10	-	14	13	15	-	-
Cons	10	14	17	14	-	-	-

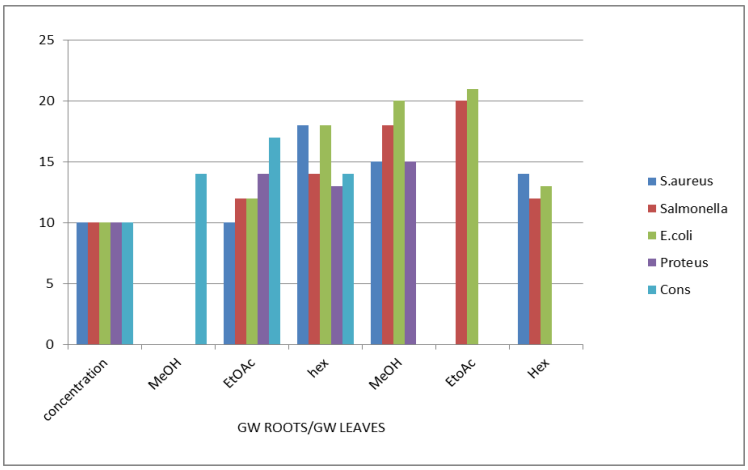

Figure 1: Comparison between inhibitory zones of methanol, Ethyl acetate and Hexane extract of G. Wallichianum against different pathogens.

Antifungal activity: Antifungal action of G. wallichianum roots and leaves crude extracts along with their respective resultant fractions were assayed by food poison technique method. The root part of G. Wallichianum crude extract showed antifungal effect of 70, 43, and 62, against, A. solani, F. oxysporum, and Collicotrichium respectively, being most effective against A. solani. shown in table 3 Figure 2 and 3 besides that the 69,44 and 48 showed antifungal effect of G. wallichium leaves against, A. solani, F. oxysporum, and Collicotrichium respectively.

Table 3: Antifungal activity of crude extract of rhizome and leave parts of geranium wallichianum on test fungal strains.

Plant		**G. Wallichianum Roots**	**G. Wallichianum leaves**
%inhibition			
Fungal strain	**Conc. mg/ml**	**Meoh**	**Eto Ac**
Alternaria Solani	100	66	46
	200	68	63
	300	70	69
Fusarium oxysporum	100	30	29
	200	36	42
	300	43	44
collectotrichum	100	48	29
	200	55	40
	300	62	48

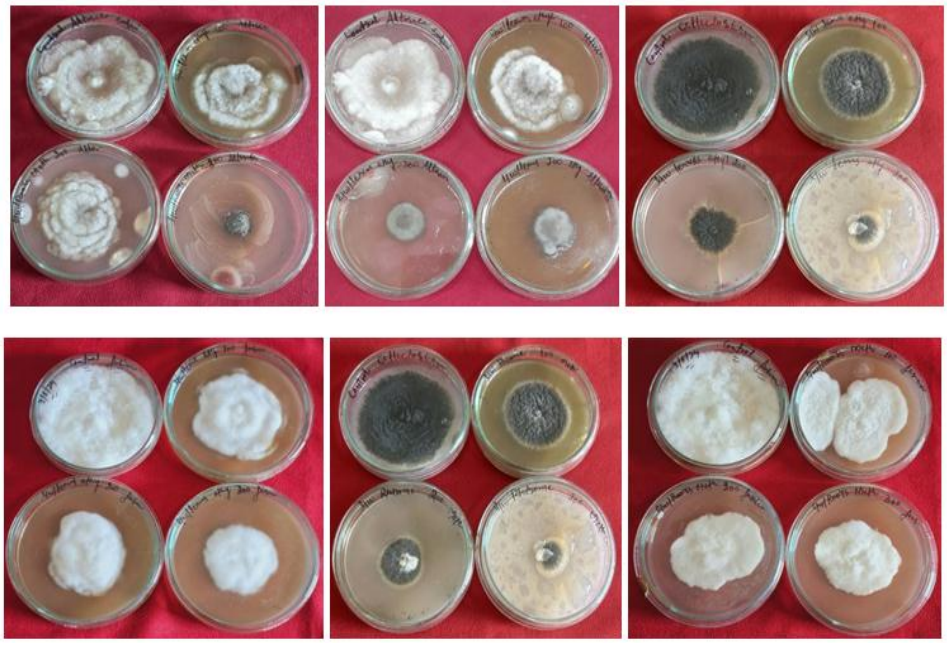

Figure 2: plant extract of roots and leaves of different concentrations showing Antifungal activity.

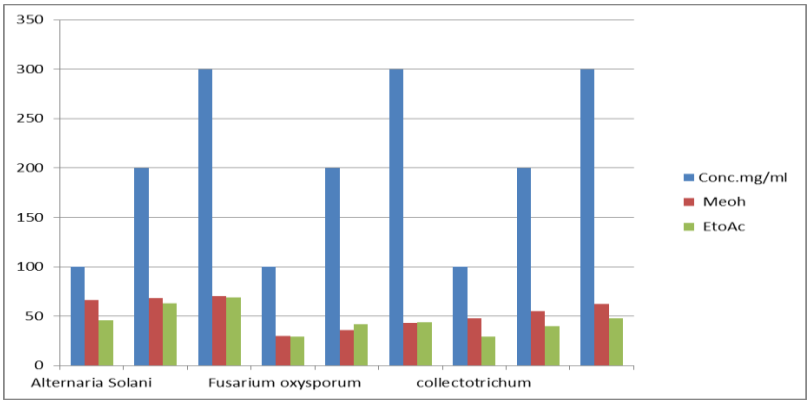

Figure 3: **Comparison between inhibitory zones of methanol, and Ethyl acetate extract of G. wallichianum against different fungal strains. GC-MS study of various G. Wallichianum solvent extracts.**

The chemical components found in the methanol, ethyl acetate, and hexane extracts of the G. wallichianum roots and leave parts are identified using the GC/MS technique. The primary components, along with their retention durations and proportional share of the overall peak area percentage, were displayed in the G. wallichianum GC/MS analysis results. In the methanol ethyl acetate and hexane of root extracts, they resulted in the identification of 6 (Table 4), 4 (Table 5), and 11 (Table 6) compounds, respectively. Besides that, in the methanol ethyl acetate and hexane of leave extracts, they resulted in the identification of 5 (Table 7), 4 (Table 8), and 5 (Table 9) respectively.

Table 4: Methanol extract of G. wallichianum roots that underwent GC-MS.

Sr. No	Compounds	Molecular formula	RT	peak area %
1	P-cymene	$C_{10}H_{14}$	3.968	3.60
2	Zidovudine	$C_{10}H_{13}N_5O_4$	5.681	6.53
3	1-(10,10-dimethyl-3,3-dioxo-3-thia-4 azatricyclo (5.2.1.0(1,5)dec-4-yl)-3-methylpent-4-en-1-one	$C_{16}H_{25}NO_3S$	6.300	1.15
4	Gamma-sitosterol	$C_{29}H_{48}O$	33.204	74.43
5	Vitamin E	$C_{29}H_{50}O_2$	38.165	10.41
6	Campesterol	$C_{28}H_{48}O$	39.379	8.00

Table 5: Ethyl acetate extract of G. wallichianum roots that underwent GC-MS.

Sr. no	Compounds	Molecular formula	RT	peak area %
1	Methyl 11,12-tetradecadienoate	$C_{15}H_{26}O_2$	4.443	1.66
2	Methyl 7,8-octadecadienoate	$C_{19}H_{34}O_2$	23.266	3.29
3	Alpha, Tocospiro B	$C_{29}H_{50}O_4$	34.443	1.27
4	Vitamin E	$C_{29}H_{50}O_2$	38.214	8.08

Table 6: Hexane extract of G. wallichianum roots that underwent GC-MS.

Sr. no.	Compounds	Molecular formula	RT	peak area %
1	Methyl 10,11-tetradecadienoate	$C_{15}H_{26}O_2$	4.017	1.06
2	2-Azido-2,4,4,6,6,8,8,-heptamethylnonane	$C_{16}H_{33}N_3$	5.649	1.96
3	2,4-decadienal(E,E)	$C_{10}H_{16}O$	7.474	2.17
4	1-(10,10-dimethyl-3,3-dioxo-3-thia-4-azatricyclo(5.2.1.0(1,5)dec-4-yl)-3-methylpent-4-en-1-one	$C_{16}H_{25}NO_3S$	12.028	1.80
5	Methyl 8,9-octadecadienoate	$C_{19}H_{34}O_2$	14.083	1.14
6	Squalene	$C_{30}H_{50}$	34.047	8.36
7	Alpha-Tocospiro A	$C_{29}H_{50}O_4$	34.409	6.14
8	Vitamin E	$C_{29}H_{50}O_2$	38.204	7.19
9	Alpha-tocopherylacetate	$C_{31}H_{52}O_3$	38.783	1.85
10	Campesterol	$C_{28}H_{48}O$	39.491	46.78
11	Stigmasterol	$C_{29}H_{48}O$	39.764	12.84

Table 7: Methanol extract of G. wallichium leaves that underwent GC-MS.

Sr. no.	Compounds	Molecular formula	RT	peak area %
1	Methyl 10,11-tetradecadienoate	$C_{15}H_{26}O_2$	4.065	1.24
2	2-Azido-2,4,4,6,6,8,8,-heptamethylnonane	$C_{16}H_{33}N_3$	7.088	3.33
3	Methyl 7,8-octadecadienoate	$C_{19}H_{34}O_2$	18.111	2.77
4	Vitamin E	$C_{29}H_{50}O_2$	38.173	3.96
5	Gamma-sitosterol	$C_{29}H_{50}O$	40.465	11.78

Table 8: Ethyl acetate extract of G. wallichianum leaves that underwent GC-MS.

Sr. no.	Compounds	Molecular formula	RT	peak area %
1	Methyl 10,11-tetradecadienoate	$C_{15}H_{26}O_2$	4.065	1.41
2	2-Azido-2,4,4,6,6,8,8,-heptamethylnonane	$C_{16}H_{33}N_3$	7.088	3.89
3	Methyl 8,9-octadecadienoate	$C_{19}H_{34}O_2$	22.524	2.48
4	Vitamin E	$C_{29}H_{50}O_2$	38.179	13.55

Table 9: Hexane extract of G. wallichianum leaves that underwent GC-MS.

Sr. no.	Compounds	Molecular formula	RT	peak area %
1	Methyl 10,11-tetradecadienoate	$C_{15}H_{26}O_2$	4.065	1.28
2	Methyl 8,9-octadecadienoate	$C_{19}H_{34}O_2$	22.525	3.68
3	1-(10,10-dimethyl-3,3-dioxo-3-thia-4-azatricyclo(5.2.1.0(1,5)dec-4-yl)-3-methylpent-4-en-1-one	$C_{16}H_{25}NO_3S$	27.670	2.26
4	Vitamin E	$C_{29}H_{50}O_2$	38.171	20.76
5	Gamma-sitosterol	$C_{29}H_{50}O$	40.478	65.65

DISCUSSION

According to studies on antibacterial activity, B. subtilis is most efficient against P.

aeruginosa. Except for the ethyl acetate fraction, which had an activity rating of 50, 38.7, and 30.3% towards S. aureus, B. subtilis, and S. flexenari, respectively, none of the rhizome extract fractions demonstrated any antibacterial activity. Using the tube dilution method, the antifungal activity of crude extracts from the rhizomes and leaves of G. wallichianum, as well as the corresponding resulting fractions, was evaluated. The rhizome-based crude extract was most efficient against F. solani, but it also shown antifungal effects against M. canis, C. albicans, C. glaberata, and F. solani of 75, 65, 60, and 55%, respectively. Except for the ethyl acetate fraction, which demonstrated activity against Candida albicans, Candida glaberata, Candida canis, and Candida the solani by 50, 50, 52, and 70%, respectively, none of the rhizome extract fractions demonstrated any antifungal effect (Muhammad Ismail et al 2011).

According to research, G. wallichianum has antibacterial activity against a variety of bacterial and fungal strains 32. Comparing the results of any research is challenging, mostly because of variations in the plant's composition and place of origin, the extraction methods used, the level of concentration of the extracted extracts, the microorganisms studied, and other factors. The antibacterial qualities of the G. wallichainum dry extracts (ethyl acetate, petroleum ether, ethanol, and methanol) acquired in the current investigation had to be assessed in order to achieve this. Several G. wallichianum extracts demonstrated significant antibacterial activity against the chosen pathogens under investigation. Significant antibacterial activity against the chosen microbial pathogens was demonstrated by the extracts of G. wallichianum from all four plants. Prior studies on the antibacterial properties of several Geranium species have also shown promising outcomes against a range of bacterial and fungal pathogens. Mir, Wajahat Rashid, et al and others 2022).

CONCLUSIONS

According to the data in this publication, G. wallichianum demonstrated a range of biological activities, including antifungal and antibacterial properties. It was discovered that the plant's roots worked better than its leaves. Therefore, the current study offers proof of the plant's numerous medicinal applications for a variety of illnesses and conditions affecting people.

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